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Evolution of polymer formation within the actin superfamily

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ABSTRACT While many are familiar with actin as a well-conserved component of the eukaryotic cytoskeleton, it is less often appreciated that actin is a member of a large superfamily of structurally related protein families found throughout the tree of life. Actin-related proteins include chaperones, carbohydrate kinases, and other enzymes, as well as a staggeringly diverse set of proteins that use the energy from ATP hydrolysis to form dynamic, linear polymers. Despite differing widely from one another in filament structure and dynamics, these polymers play important roles in ordering cell space in bacteria, archaea, and eukaryotes. It is not known whether these polymers descended from a single ancestral polymer or arose multiple times by convergent evolution from monomeric actin-like proteins. In this work, we provide an overview of the structures, dynamics, and functions of this diverse set. Then, using a phylogenetic analysis to examine actin evolution, we show that the actin-related protein families that form polymers are more closely related to one another than they are to other nonpolymerizing members of the actin superfamily. Thus all the known actin-like polymers are likely to be the descendants of a single, ancestral, polymer-forming actin-like protein.

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BACKGROUND

Actin was one of the first biological polymers to be biochemically characterized. It was first extracted from skeletal muscle tissue by Halliburton in 1887, who referred to it as “myosin ferment” due to its ability to coagulate in the presence of the motor protein, myosin (Halliburton, 1887). In 1942, Straub purified monomeric actin, opening up the possibility of studying polymer behavior in vitro (Straub, 1942). Later, Huxley and Hanson showed that actin filaments are one of the major components of contractile muscle sarcomeres (Hanson and Huxley, 1953), and Pollard identified actin as the thin filaments present in the contractile cytoplasm of *Acanthamoeba* (Pollard et al., 1970). Since then, work by many researchers has

elucidated the ultrastructure, regulation, dynamics, and function of actin filaments from a wide range of eukaryotes, in which it functions in concert with other cytoskeletal filaments to control cellular organization. Although this work revealed the ubiquity of eukaryotic actin, actin's place as one of the distinguishing features of eukaryotes changed with the discovery of similar filaments within bacteria, archaea, and mobile genetic elements (Bork et al., 1992; Møller-Jensen et al., 2002). As this work made clear, actin is in fact a single member of a much larger superfamily of proteins that are present in organisms across the tree of life. Here we refer to members of this superfamily as “actins” if they cluster with canonical eukaryotic actins in phylogenetic analyses, while we call other proteins in the superfamily “actin-related” polymers or “actin-related” proteins.

Strikingly, many members of the superfamily have been shown to form dynamic polymers. Despite differing widely in their higher-order structures and assembly kinetics, these polymers carry out a number of conserved functions across the domains of life: they control the shape of both eukaryotic and many prokaryotic cells; are common, conserved components of the cell division and chromosome segregation machinery (Mishra et al., 2014); and control the dynamic internal organization of the cytoplasm in eukaryotes and some prokaryotes. In addition, the actin superfamily also includes a number of other proteins that do not form polymers, including

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chaperones such as Hsp70/DnaK and enzymes such as hexokinase and glutamate mutase (Hurley, 1996).

The structural similarity between these diverse proteins raises a number of questions. How are the actin-like polymers and nonpolymerizing actin-like enzymes related? How likely is it that a diverse set of actin-related enzymes form polymers? Did polymerization arise once, or multiple times, during evolution? What common features do these evolutionarily distant proteins share that enables them to form polymers, and what features are modified across evolution to change their filament superstructure, polymerization dynamics, and cellular function? In this review, we look at the evolution of actin-like filament structure and function across the tree of life. In doing so, we consider two reciprocal hypotheses: 1) that the self-assembly of actin filaments arose only once in evolution—that is, all extant actin-like filaments are descended from a single ancestral polymer, or 2) polymerization arose multiple times within the actin superfamily as the result of modifications in the structure of one or more monomeric ATPases with an actin-like fold.

In exploring the evolution of actin-like proteins, we begin by examining their structure and functions across systems. We then look at the phylogenetic tree of actins and related proteins to test these alternative hypotheses and determine the origin of polymerization within the actin superfamily.

THE STRUCTURE OF ACTIN AND ACTIN-LIKE FILAMENTS

Actin is one of the most ubiquitous and highly conserved proteins in eukaryotes, being 91% identical in sequence between *Caenorhabditis elegans* and *Homo sapiens*. It is also among the set of proteins that appear to have been inherited from the last common ancestor of all eukaryotes. Nevertheless, the sequences of actins differ more widely in some eukaryotic lineages, for example, in plants (McDowell et al., 1996). Thus the high level of sequence conservation between actins across eukaryotes may not reflect a fundamental limit in actin's ability to tolerate sequence changes while still forming dynamic filaments. Instead, this conservation may reflect other complex constraints, for example, those imposed by the need to preserve interactions with a wide set of actin-binding proteins, or those due to actin's high levels of expression in eukaryotes (Pál et al., 2001). In addition, when we consider the prokaryotic members of the actin family, the degree of variation that can be tolerated within the context of an actin-like polymer becomes clear. These distant relatives have sequences so divergent that they were only first identified using a motif built from an alignment of actin, Hsp70, and hexokinase (Bork et al., 1992).

The first low-resolution structure of the actin monomer was obtained by Kabsch, Mannherz, and Suck in 1985, and the same team produced the first atomic model in 1990 with the help of Pai and Holmes (Kabsch et al., 1985, 1990). With the structure in hand, it became immediately apparent that, despite their low sequence similarity, actin and hexokinases shared the same fold (Steitz et al., 1978). At the heart of actin's two globular domains is a nucleotide-binding pocket that binds and hydrolyzes ATP. Importantly, the structure of the monomer is profoundly changed by nucleotide binding and subtly altered by nucleotide hydrolysis from ATP to ADP. As a result, ATP hydrolysis and subsequent phosphate release lead to movement of the two domains of the protein relative to one another (Korn et al., 1987; Orlova and Egelman, 1992; Murakami et al., 2010). It is this link between nucleotide binding, ATP hydrolysis, and changes in monomer structure that are likely to make actin-related enzymes a good starting point for the formation of actin-like filaments.

Allostery and monomer contacts within an actin-like filament

When embedded within a filament, actin-related monomers make extensive contacts with one another. These include head-to-tail contacts between monomers within individual protofilaments and contacts between the two filament strands (Figure 1). In most actin-like polymers, the location of the head-to-tail monomer contacts within the protofilaments are conserved: subdomain Ia in one monomer interacts with residues in subdomain Ib in the next monomer in the series, while subdomain IIa interacts with subdomain IIb in a similar way (Figure 1C). Even in FtsA, which is missing subdomain Ib, the longitudinal contacts appear to be conserved; an insertion into subdomain Ia takes the place of subdomain Ib within a presumed polymer (Szwedziak et al., 2012; Ozyamak et al., 2013b). Thus these contacts may be essential across actin-related proteins to enable the formation of dynamic filaments, because nucleotide binding shortens the distance separating subdomains Ib and IIb, matching the distance between subdomain Ia and IIa (van den Ent et al., 2014) (Figure 1).

As a result of these contacts, changes in the conformation of individual monomers that result from ATP hydrolysis and phosphate release can be felt by neighboring monomers in the filament. As well as making actin an excellent chassis for use in the generation of cytomotive filaments (filaments that generate force via polymerization or depolymerization), this type of monomer–monomer communication may enable actin-related proteins to act as allosteric enzymes with high cooperativity. It is therefore formally possible that some members of the enzyme/chaperone actin subfamilies form polymers. This would not be without precedent, as other enzymes have been shown to assemble into filaments that regulate their activity: one of the best examples being the non-actin related protein, CTP synthetase (Barry et al., 2014). In this case, polymerization can either activate or inhibit CTP synthetase activity depending on the organism (Noree et al., 2010; Lynch and Kollman, 2016). Moreover, several metabolic enzymes in yeast have been shown to form pH-dependent polymers in response to starvation (Shen et al., 2016). For most actin-related enzymes, however, this hypothesis remains to be tested.

Variation in the structure of actin-related filaments

Even though all members of the actin superfamily appear similar at the monomer level, they differ greatly in their filament structure. Much of this variation comes from changes in the lateral contacts between protofilaments (van den Ent et al., 2014). This can alter helical pitch and filament handedness and can determine whether filament strands lie parallel or antiparallel to one another (Figure 1C). Because of this, filaments built from monomers with a similar fold can have very different properties.

In the case of eukaryotic actin, two parallel filament strands twist around one another like rope to form a right-handed helical filament that is both chiral and polar. Many of these structural features of the polymer are essential for actin's function. For example, filaments must be polar to be used as a substrate for directed molecular motors, like myosin (Schuh, 2011). However, the protofilament polarity, pitch, handedness, and degree of stagger between protofilaments varies across polymers within the actin superfamily. Moreover, as highlighted in the following sections, these structural differences have significant consequences for polymer dynamics and function (see Figure 1).

MreB polymers are involved in bacterial cell wall synthesis and maintenance of prokaryotic rod shape. MreB forms two-stranded, antiparallel, nonhelical filaments, with no stagger between the

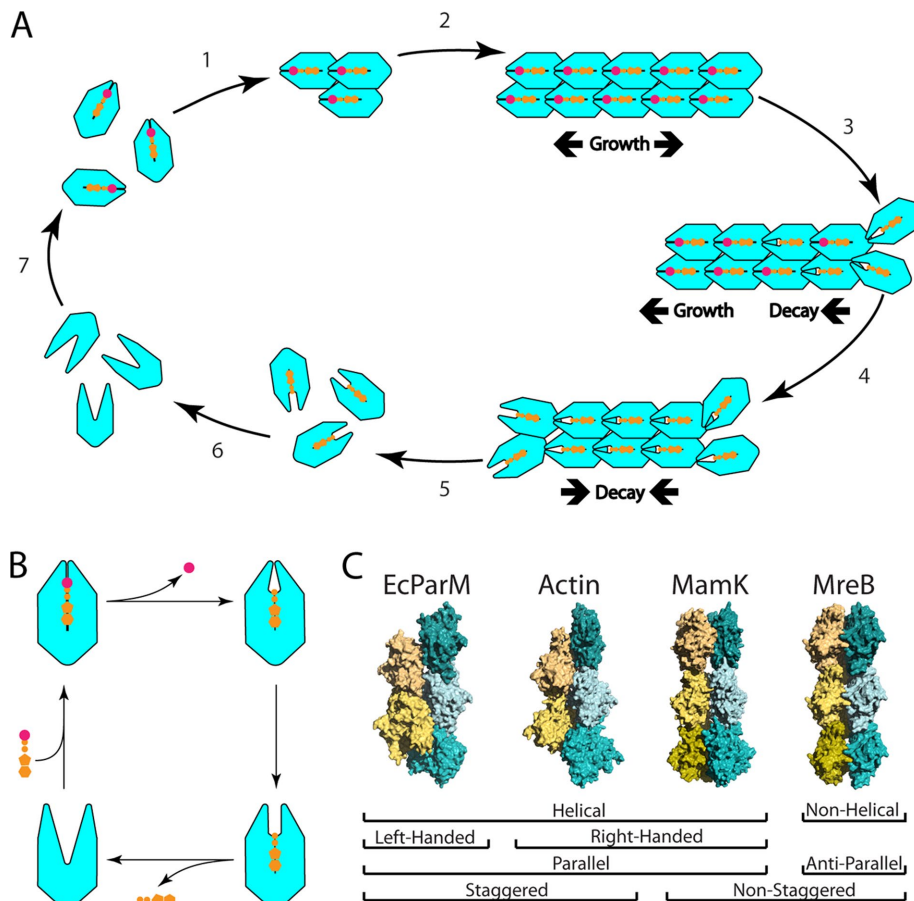


FIGURE 1: Polymer formation is a repeated feature within the actin superfamily. (A) Polymerization of actin homologues requires the formation of a filament nucleus (1). Once a nucleus has formed, filament elongation is rapid (2). Once monomers incorporate into a growing filament, they begin to hydrolyze ATP (3) until the filament end(s) is(are) composed of ADP-bound monomers (4). Filaments initially grow in both directions. Under certain conditions, some filaments can grow from one end and shrink from the other (a process referred to as treadmilling). Eventually, lower affinity between ADP-bound monomers allows for filament disassembly from the ends (5). ADP then dissociates from ADP-bound monomers (6), which then rapidly rebind ATP (7). (B) ATP hydrolysis, phosphate release, ADP/ATP exchange are associated with changes in monomer conformation that influence filament architecture and actin on/off rates. (C) Among actin-like filaments, the contacts within individual protofilaments vary little. By varying lateral contacts between protofilaments, different filament structures with different properties and behaviors can be generated. A subset of these structures is displayed here. *E. coli* ParM is a parallel, left-handed, helical filament whose protofilaments are staggered (PDB ID: 5AEY) (Bharat et al., 2015). Actin is a parallel, helical filament whose protofilaments are staggered (PDB ID: 4A7N) (Behrman et al., 2012). MamK filaments are parallel, helical filaments whose protofilaments are not staggered (PDB ID: 5LJV) (Löwe et al., 2016). MreB is a nonhelical, antiparallel filament whose protofilaments are not staggered and that has an intrinsic curvature (PDB ID: 4CZJ) (van den Ent et al., 2014).

subunits in each protofilament (van den Ent et al., 2014). The lack of a twist is essential to MreB function in two ways. First, because MreB associates with the membrane through a single face, the absence of filament twist enables adjacent monomers to form tight contacts with the membrane. Furthermore, because MreB lacks a helical twist, MreB filaments can sustain an intrinsic curvature—something that appears to be crucial for its function in the regulation of bacterial cell shape (Salje et al., 2011; van den Ent et al., 2014).

MamK organizes the cellular distribution of magnetic organelles (magnetosomes) within magnetotactic bacteria. MamK filaments form parallel helical filaments like actin but with no stagger between the subunits, leaving an open cavity between the protofilaments

(Ozyamak et al., 2013a; Löwe et al., 2016; Bergeron et al., 2017). This raises some interesting questions about how these structural differences influence MamK polymer dynamics, the interaction of the polymer with magnetosomes, and how the lack of stagger affects the mechanical properties of MamK filaments.

ParM from the *Escherichia coli* R1 plasmid (EcParM) forms parallel, twisted filaments, but unlike actin, has a left-handed twist. In contrast, ParM from the *Bacillus thuringiensis* pBMB67 plasmid (BtParM), forms two-stranded, supercoiled, antiparallel, helical filaments. Interestingly, in the presence of its DNA adaptor protein (ParR), these filaments associate to form a four-stranded nanotubule with an open core; a structure reminiscent of microtubules (Jiang et al., 2016b). While this structure's mechanical properties have not been studied, it is likely that these filaments are stiffer than two-stranded filaments, again reminiscent of the mechanical properties of microtubules or of stiff fascin-cross-linked actin bundles seen in many eukaryotic cells (Jansen et al., 2011; Takatsuki et al., 2014).

Variation in filament kinetics

Actin-like polymers also differ widely from one another in their dynamics. Changes in kinetic parameters (e.g., nucleation rate, monomer on/off rate, ATPase activity, phosphate-release kinetics) can yield polymers with different length distributions, stability, and polymerization and depolymerization rates. Importantly, however, because polymer dynamics are sensitive to changes in amino acid sequence that cause little or no change in secondary and tertiary structure, this may not be evident from a simple structural analysis of the polymer. Changes in kinetics can also be brought about by associated regulators. Thus, in eukaryotes, a small number of actins are used to perform a wide variety of cellular tasks through the action of a large number of actin-binding proteins that modify different kinetic and/or structural parameters of the monomer/polymer. This tunability enables cells to exert

dynamic local control over the actin cytoskeleton to determine where actin filaments form, how fast they elongate, and for how long they persist (Pollard et al., 2001). Cross-links between actin filaments further expand this repertoire to enable networks with different filament organization (Mullins et al., 1998; Jacinto and Baum, 2003).

In eukaryotes, much of the control is exerted at the level of nucleation. This is because actin is a so-called nucleation-condensation polymer, that is, the affinity of a monomer for another monomer is much lower than its affinity for a growing filament or a filament nucleus (Lal et al., 1984; Mullins et al., 1998). This is both a natural consequence of monomers having multiple contacts in the context

of a filament and a useful property, because it disfavors the spontaneous nucleation of filaments even in the presence of high concentrations of the monomer. As a result, nearly all actin-like polymers whose assembly dynamics have been elucidated have been found to be nucleation-limited. However, relative to eukaryotic actin, the nucleation rate tends to be much faster in the case of bacterial actins. For example, the nucleation rate of EcParM is 200 times faster than eukaryotic actin, even though filaments elongate at a similar rate (Garner *et al.*, 2004). Similarly, the plasmid-segregating actin AlfA from *Bacillus subtilis* also assembles via a rapid, yet nucleation-limited pathway (Polka *et al.*, 2009). In these cases, the ability to undergo spontaneous polymerization at a suitable site may do away with the need for a dedicated nucleator. Interestingly, there are eukaryotic actins within parasites such as *Toxoplasma gondii* that assemble isodesmically (the affinity of a monomer for another monomer is equal to the affinity between a monomer and a filament end), thus obviating the need for a nucleator (Skillman *et al.*, 2013). These actins have been reported to exhibit atypical lateral filament contacts (Vahokoski *et al.*, 2014) and form short microfilaments just beneath the cell membrane.

Following polymerization, the behavior of a filament will depend on the rates of ATP hydrolysis and phosphate release. As ATP-bound monomers are incorporated into actin filaments, bound ATP is hydrolyzed such that the ends of the filament tend to be composed of ATP-actin as long as the filament continues to grow (Carlier *et al.*, 1984) (Figure 1). This in turn affects polymer dynamics because, for eukaryotic actin, the ADP-bound form has an off rate at the minus end that is much higher than that of ATP actin (Pollard and Borisy, 2003). This, together with asymmetries in the on/off rates at each end of the filament, can lead to treadmilling, whereby monomers flux through a filament of constant length as the filament grows via monomer addition at its (+) end at the same rate as it shrinks via monomer loss from its (–) end (Kirschner, 1980). Other actin-like polymers display this behavior. These include the plasmid segregators Alp7a and AlfA (Derman *et al.*, 2009; Polka *et al.*, 2009) and MamK, which treadmills to distribute magnetosomes between daughter cells (Toro-Nahuelpan *et al.*, 2016).

Actin-related filaments can also exhibit dynamic instability, the rapid transitioning between states of polymerization and depolymerization—a key feature of microtubule dynamics (Mitchison and Kirschner, 1984). While eukaryotic actin does not exhibit dynamic instability (for a possible exception see Husson *et al.*, 2011), it is observed in the plasmid-segregating polymers EcParM and Alp7a (Garner *et al.*, 2004; Drew and Pogliano, 2011). Although there are exceptions to this rule, for example, AlfA (Polka *et al.*, 2014), this suggests that evolution may have converged on a common solution that allows the efficient “search and capture” of DNA by cytoskeletal elements across different systems (Mitchison and Kirschner, 1984).

At the same time, there are many clades of structurally similar enzymes that do not appear to form polymers. These include the many “actin-related proteins” (Arps) in eukaryotes. Although, in the case of the Arp2/3 complex, the actin-related proteins in the complex have lost their ability to form filaments themselves, the conformational change associated with their ATPase activity is still used to cause allosteric changes required for the protein to function. The nucleotide state determines whether the dimer is in an open or closed state, which regulates its ability to seed new actin filaments (Goley *et al.*, 2004; Dalhaimer *et al.*, 2008) and its affinity for other factors (Ti *et al.*, 2011). It remains to be seen whether there are similar nonpolymerizing proteins within the bacterial family of “actin-like proteins” (Alps) (Derman *et al.*, 2009).

THE FUNCTIONS OF ACTIN-LIKE PROTEINS

Having examined the structure and dynamics of actin and actin-related proteins and polymers, we next explore the conserved roles of the different actin-like polymers: focusing on their roles in the regulation of cell shape, organization, and division across kingdoms.

Bridging long-length scales

One of the primary cellular roles of several different actin-like polymers is to bridge length scales. As both actin and ParM filaments have a persistence length of ~10 microns (Gittes *et al.*, 1993; Choi *et al.*, 2008), cells beneath this size can use these relatively rigid filaments as a means to establish and/or read out long-range order (Theriot, 2013). To exert spatial control over greater distances, short filaments can be cross-linked together or coupled to stiffer cellular components (e.g., microtubules). In large eukaryotic cells, these actin tracks are used to traffic cellular components over long distances, a transport mediated by processive myosin motors moving along parallel actin filament bundles (Boldogh and Pon, 2006; Massarwa *et al.*, 2009; Schuh, 2011; Rouso *et al.*, 2013). Conversely, at the small length scales found in most prokaryotic cells, cargoes such as plasmids are trafficked to cell poles by associating with the growing filament ends. (Drew and Pogliano, 2011; Gayathri *et al.*, 2012; Polka *et al.*, 2014; Toro-Nahuelpan *et al.*, 2016) This strategy obviates the need for linear stepping motors, molecules that have yet to be discovered in prokaryotes.

In this context, filaments can be used to organize cell space in several ways. When elongating within a confined space with a simple geometry (like a cylinder), long and stiff filaments will orient along the cell's long axis, thus growing to the ends of the cell (Møller-Jensen *et al.*, 2003; Garner *et al.*, 2007). Likewise, in the filamentous fungus *Ashbya gossypii*, cell wall material is trafficked to the growing tips of the hypha along bundles of actin filaments (Schmitz *et al.*, 2006), and in budding yeast, actin-mediated transport plays a critical role in moving material from the mother to the emerging bud (Yin *et al.*, 2000; Rouso *et al.*, 2013; Westermann, 2014; Knoblauch and Rachubinski, 2015). Thus, actin filaments perform a vital function in cell shape control in both fungi and plant cells by defining the sites of cell wall insertion, while actin-like (e.g., MreB) filaments perform a similar role in generating the form of rod-shaped bacteria (see below) (Dye *et al.*, 2005).

Interestingly, because animal cells have a flexible form, actin performs a similar role in the regulation of animal cell shape in a different way. Here actin filaments are often cross-linked to generate higher-order networks, whose material properties can be precisely tuned to set the physical properties of cells and tissues. Thus, in animal cells, the actin meshwork that underlies the plasma membrane provides the plasma membrane with vital structural support and with a capacity to undergo dynamic changes in shape, for example, to form protrusions, retraction fibers, and phagocytic cups (Clerc and Sansonetti, 1987; Cramer and Mitchson, 1993; Nemethova *et al.*, 2008). Similarly, actin helps to define the material properties of both the nucleoplasm (Grosse and Vartiainen, 2013) and the cytoplasm (Gordon *et al.*, 1976).

Force generation through polymerization dynamics

By leveraging the release of free energy associated with ATP hydrolysis, cells can use the polymerization of filaments to exert force. In the context of actin networks, force production has been posited to occur in a variety of ways (Dmitrieff and Nédélec, 2016). One of the simplest mechanisms is the Brownian ratchet (Theriot, 2000), where ATP hydrolysis within filaments holds the system far from equilibrium, and thus constantly polymerizing filament ends iteratively rectify

fluctuations in the position of a load. Similarly, in *EcParM*, insertional polymerization at *ParM* filament ends causes directional plasmid movement (Garner *et al.*, 2007). It has also been proposed that cells can use the depolymerization of actin filaments to generate pulling forces (Jégou *et al.*, 2013). Moreover, in the presence of multivalent motors, like myosin II, which can bind multiple actin filaments at the same time, the forces exerted by the motors can cause an actin network to contract or expand (Huxley and Niedergerke, 1954). However, because individual filaments tend to buckle under compressive forces, this break in the symmetry usually ensures that cross-linked actin networks contract under the influence of myosin-based motors (Lenz, 2014).

Finally, actin-related filaments can also function to sense local curvature, for example, through their association with membranes. The best example of this is *MreB*. *MreB* filaments are highly curved (Salje *et al.*, 2011; van den Ent *et al.*, 2014). This may enable *MreB* filaments to read out the differences in the local curvature of the bacterial membrane, causing them to align along the circumference of the cell, perpendicular to the straight, long cell axis. This orientation would guide the associated cell wall synthetic machinery so that new cell wall material is laid down in hoops, ensuring that bacteria retain their rod shape against the internal pressure (Chang and Huang, 2014). *MreB* filaments have also been shown to show a small, but significant increased localization at points of negative Gaussian curvature (Ursell *et al.*, 2014). Interestingly, an archaeal actin, crenactin, is present in rod-shaped crenarchaea, but is absent from many more pleomorphic archaea (which can change their shape in response to environmental cues), suggesting that it could perform a similar function in guiding archaeal cell shape (Ettema *et al.*, 2011), even though the shape of these cells is supported by an external S-layer rather than a cell wall.

Cell division

Cell division is one of the most basic cellular processes to rely on force production and on long-range intracellular trafficking. Actin, along with myosin II, is an essential component of the cytokinetic ring in eukaryotes (Wong *et al.*, 2002; Miller, 2011). The contractile forces generated by myosin II within the contractile ring cause actin filaments to slide past one another (Miller, 2011). At a larger scale, similar rings, encompassing many cells, provide the contractile forces required for wound healing and tissue morphogenesis (Jacinto and Baum, 2003). Importantly, in these cases, the tension generated around the ring can function to drive changes in cell/tissue shape and/or to provide a template with a simple uniform geometry that can then be used to guide and organize external processes such as cell wall synthesis in yeast or dorsal closure in fly embryos (Jacinto *et al.*, 2002).

Interestingly, bacterial actins are also used in prokaryotic cell division. In this case, actin-related proteins aid division without the aid of a myosin-like motor. The bacterial actin *FtsA* polymerizes along with the tubulin homologue *FtsZ* at the septum of dividing cells (Ma *et al.*, 1996). Both *FtsA* and *FtsZ* treadmill around the division plane (Loose and Mitchison, 2014; Bisson-Filho *et al.*, 2017), and treadmill controls the location and activity of the associated cell wall synthetic enzymes. It is unclear whether *FtsA/FtsZ* treadmill is responsible for generating the forces for cell division, or whether the filaments themselves bend the membrane. Alternatively, the composite *FtsA/FtsZ* ring may simply act as a scaffold guiding the movement of enzymes that insert septal cell wall material (Egan and Vollmer, 2015)—as has been proposed for actomyosin rings in eukaryotes with a cell wall (Otegui and Staehelin, 2000; Chang, 2017), where actin filament turnover likely contributes to ring homeostasis (Chew *et al.*, 2017).

During cell division, actin-related proteins also drive the segregation of large cellular objects (Jiang *et al.*, 2016a). For example, *MamK* is responsible for distributing magnetosomes along the long cell axis (Komeili *et al.*, 2006) and partitioning them into daughter cells (Toro-Nahuelpan *et al.*, 2016), while bidirectionally growing *ParM* filament bundles segregate low-copy plasmids to ensure each daughter cell inherits a copy. Similar end-finding strategies have been observed for other actin-related plasmid segregators, each displaying subtle variation in its filament kinetics and regulation (Derman *et al.*, 2009; Polka *et al.*, 2009). Thus actin-related proteins are an essential component of the cell division machinery across kingdoms (Balasubramanian *et al.*, 2012).

Macromolecular scaffolds

Finally, actin and actin-related filaments can serve as scaffolds for larger complexes. An example of this is the dynactin complex. This complex links vesicles to the motor dynein and contains short filaments of actin and *ARP1* (Urnavicus *et al.*, 2015). This filament helps to orient and adapt various organelles for microtubule-mediated transport (Gill *et al.*, 1991; Plamann *et al.*, 1994). The polarity of this actin-like filament ensures that dynein engages with its cargo in the proper orientation. This type of role for short filaments may be more widespread, as actin and ARPs have recently been suggested to play a similar function in the regulation of eukaryotic transcription (de Lanerolle, 2012).

THE EVOLUTION OF ACTIN-FAMILY PROTEINS

Structural similarity and shared ancestry among actin-like proteins

To what extent do the related structure and function of prokaryotic and eukaryotic actins reflect the action of selection, and to what extent do they reflect the constraints imposed by common ancestry? To answer this, we next take a look at the evolution of the actin-related proteins.

Because primary sequence evolves faster than tertiary structure (Illergård *et al.*, 2009), structural similarities can preserve evidence of common evolutionary ancestry even after sequences have diverged beyond recognition. At the same time, proteins with different evolutionary origins may converge on (that is, evolve toward) similar structures in response to selection for a particular function. Given the diversity of functions performed by actin-like proteins across the tree of life, it therefore seems reasonable to ask whether their shared fold is the result of convergence or common ancestry.

Here, to provide a formal test of the common ancestry of all protein families sharing the actin fold, we built hidden Markov models (HMMs) from sequence alignments of each family and aligned these profiles using the *halign* program in the HH-suite package (Remmert *et al.*, 2011). These comparisons are more sensitive than a standard BLAST search because they incorporate information about the evolutionary conservation of residues within each protein family. Because they are still based on comparisons of primary sequences, they also provide a way to test hypotheses of common ancestry or convergence. This is done by determining whether the levels of sequence identity between two families are better than those expected by chance, giving evidence for descent from a common ancestor or of a common structural constraint. The results of this analysis (Supplemental Figure S2) provide strong evidence of significant sequence similarity, and therefore common ancestry, among many of the actin-like protein families. This is consistent with their structural similarities and suggests that filament-forming and monomeric actin-like families share a common ancestor. Note that a lack of significant similarity does not rule out an evolutionary

relationship between two families, particularly in light of the structural similarities among all these families.

Evolution of polymerization in the actin superfamily

Having established a likely common ancestry for all known actin-like proteins, we next inferred a phylogenetic tree to determine the relationships among the subfamilies and to assess the number of times that polymerization has evolved. A prerequisite for this phylogenetic analysis is sufficient similarity among sequences to permit alignment. Thus we limited our tree inference to the set of actin-like families for which all pairs show significant pairwise sequence similarity (with an E-value cutoff of $< 10^{-5}$): a set that includes the polymers actin, MreB, FtsA, ParM, and PilM; and the enzymes DnaK, type II secretion protein L, diol dehydratase reactivase, peptidase M22, BcrAD-BadFG, glutamate mutase, 2-hydroxyglutaryl-CoA-reductase, EutA, FGGY carbohydrate kinase, AnmK, GDA1-CD39, and PPX GPPA phosphatase (Supplemental Tables S1 and S2). HMMs built from representative sequences for each of these subfamilies were aligned against one another using the E-INS-i mode in mafft (Katoh and Standley, 2013), and poorly aligning positions were identified and removed using trimAl (Capella-Gutiérrez et al., 2009). Bootstrapped maximum-likelihood phylogenies were inferred under the LG+C20+G+F model in IQ-Tree (Nguyen et al., 2015), providing a model that accommodates variation in exchange rates among amino acids (LG) (Le and Gascuel, 2008; Quang et al., 2008), variation in equilibrium composition frequencies across sites (C20) (Quang et al., 2008), and variation in evolutionary rates across sites (G, discrete gamma distribution) (Yang, 1994).

The maximum-likelihood phylogeny (Figure 2) resolves the filament-forming actin families into a single clan (Wilkinson et al., 2007) or lineage on the tree, so the split occurring between polymers and monomers receives good (93) bootstrap support. This result strongly suggests that polymerization evolved once during the evolution of actins and that all filament-forming actins are descended from an ancestral polymer-forming, actin-like protein.

Given the lack of an obvious outgroup, it is impossible to unambiguously root the tree of actins and actin-like proteins. It nonetheless seems reasonable to assume that polymers would evolve from monomers. Interestingly, however, the monomeric actins do not form a single lineage in our tree, rather, the Hsp70/DnaK family of molecular chaperones is found nested within the polymer clade, separated from the other monomeric actins with good bootstrap support. Although our unrooted tree does not enable us to exclude the possibility that the root of the entire actin family lies within one of these two groups, the high level of sequence similarity between Hsp70/DnaK and the filament-forming actins (Supplemental Figure S2) is, in our view, more readily explained by an origin of this molecular chaperone via a secondary loss of polymerization. Consistent with this view, it is interesting to note that, while none of the actin family polymers can be confidently mapped to the last universal common ancestor, some of the monomeric forms—including benzoyl-CoA reductase and hydantoinase—are broadly distributed in modern bacteria, archaea, and some eukaryotes (Finn et al., 2016) (Supplemental Table S1), indicating the radiation of the monomeric actins may predate the deepest splits in the tree of life.

The prokaryotic origins of eukaryotic-type actin

Given that eukaryotes appear to have arisen from the symbiosis of cells from both bacterial and archaeal lineages, both of which contain actin-like proteins, the origin of eukaryotic actin has long remained a question in the field. Fortunately, this mystery now

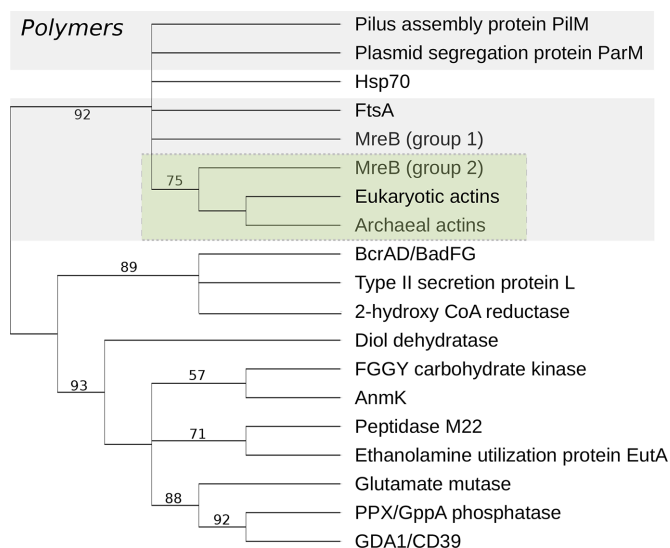


FIGURE 2: Evolutionary relationships among members of the actin superfamily. The phylogeny was inferred under the LG+C20+G+F model in IQ-Tree (Nguyen et al., 2015), and branch supports are maximum-likelihood bootstrap values. All polymer-forming actins cluster together in the tree, suggesting that the capacity to form filaments arose once during the evolution of the actin fold. Interestingly, the molecular chaperone DnaK/Hsp70 falls within the polymer lineage, suggesting that it may have evolved from an ancestral polymer-forming actin by loss of polymerization. The actin-like proteins of Crenarchaeota and the Asgard archaea (indicated as “Archaeal actins”) are the closest prokaryotic relatives of bona fide eukaryotic actins, consistent with a close relationship between the Asgard superphylum and the archaeal host cell for the mitochondrial endosymbiont (Braun et al., 2015; Spang et al., 2015; Zaremba-Niedzwiedzka et al., 2017). The archaeal actins and the eukaryotic actins together form a lineage that is most closely related to the cell shape-determining protein MreB, found in rod-shaped bacteria. Our phylogenetic analysis suggest that actins are nested within the diversity of MreB proteins (green box), although statistical support for the specific relationship is low (Supplemental Figure S1). We have depicted the actin tree as unrooted: the divergences between superfamily members are ancient, with some likely occurring before the time of the last universal common ancestor. It nonetheless seems reasonable to suppose that the polymer-forming actins evolved from an ancestral monomer, suggesting that the root may lie somewhere among the monomeric actins. Among modern actin-like proteins, only two proteins, both monomeric—benzoyl-CoA reductase (BcrAD/BadFG) and hydantoinase (not depicted in this tree, due to high levels of sequence divergence)—are broadly distributed in bacteria, archaea, and among some eukaryotic lineages and may represent good candidates for the oldest extant members of the superfamily; both also perform functions that may have been important during the evolution of early life. A complete version of this schematic tree is available in the Supplemental Material (Supplemental Figure S1).

appears to have been resolved. Recent discoveries have identified close homologues of the eukaryotic-type actins in the genomes of members of the TACK and “Asgard” superphyla (Braun et al., 2015; Spang et al., 2015; Zaremba-Niedzwiedzka et al., 2017), the lineages of archaea that appear to be most closely related to eukaryotes. Furthermore, functional studies of “crenactin” filaments from *Pyrobaculum* indicate that they are nearly identical to the filaments formed by eukaryotic actin (Izore et al., 2014; Braun et al., 2015), and at least one gelsolin-like protein (arcadin-2) seems to act

on crenactin polymers in a manner similar to the way in which gelsolin acts on eukaryotic actin (Izore *et al.*, 2016). While functional studies have not yet been performed on Asgard actins, both crenactins and Asgard actins cluster with the actins of canonical eukaryotes in our phylogenetic analysis (Figure 2). Thus direct evolutionary precursors of eukaryotic actin appear to have first evolved in the archaeal ancestors of eukaryotes. It is also interesting to note here that the metagenomic assembly of *Lokiarchaeum*, the first member of the Asgard group to be discovered, also encodes potential homologues of the actin modulators profilin and gelsolin. If these proteins prove to modulate the polymer kinetics of a Lokiarchaeal actin *in vitro*, it would suggest that at least some of the functions and regulatory mechanisms of eukaryotic actins evolved before the split between eukaryotes and archaea. While the cellular function of these archaeal actin homologues remains a matter of speculation, this is an exciting time for research into the origin of the eukaryotic cytoskeleton. It is clear that much more functional information is needed to answer the following questions: Do Asgard actins form actin-like filaments? If they do, what are the kinetic properties of archaeal actin polymers? Do the predicted actin modulators present in the *Lokiarchaea* modulate Lokiarchaeal actin filaments? Does crenactin perform similar cellular functions to that of eukaryotic actin?

CONCLUSIONS AND PERSPECTIVES

In this review, we have examined the similarities in the structure and function of actin-like proteins across the tree of life. Importantly, our evolutionary analysis suggests that the known polymer-forming actin-like proteins from bacteria, archaea, and eukaryotes have all arisen from a single ancestral polymer-forming protein. This makes actin and its relatives an ancient protein (Doolittle, 1995). Importantly, much of the structural variation that appears to underpin changes in the biological function of actin-related proteins across domains is based on changes in lateral contacts between protofilaments (van den Ent *et al.*, 2014), yielding changes in twist, packing, curvature, and polarity. In addition, it is clear that there are wide differences in the dynamics of polymer formation and disassembly across the clade. More work needs to be done to survey the variations in kinetic properties of different actin-like subfamilies (MreB, actin, MamK, ParM, etc.) to understand how subtle differences in the context of a similar fold can lead to dramatically different behaviors. For the one well-studied case of ParM, a weakening of the cross-protofilament contacts occurs when filaments are ADP bound (Bharat *et al.*, 2015). In addition, our analysis raises the question of whether Hsp70/DnaK can form polymers or has lost that capability during evolution.

It seems remarkable that so many of the cytomotive filaments used to generate intracellular forces, to order cellular organization, and to drive cell division across living systems are based on this single scaffold. This reflects the role of evolution as tinkerer (Jacob, 1977). It may also point to the special properties of the actin fold within the polymer-forming enzymes. Actin couples ATP hydrolysis and phosphate release to large-scale changes in conformation, altering monomer–monomer contacts in the context of a filament, giving rise to polymer dynamics and the capacity to do work. Of course, there are instances in which biological polymers appear to have evolved from nonpolymerizing proteins. These include CTP synthetase, sickle-cell hemoglobin, and MSP from *C. elegans* (Edelstein *et al.*, 1973; Italiano *et al.*, 2001; Ingerson-Mahar *et al.*, 2010). However, sickle-cell hemoglobin forms long, static, polar filaments that distort cells, and CTP synthase and MSP form non-polar filaments. CTP synthase polymerization appears to inhibit the

enzyme activity in most cases, and MSP polymerization is not directly coupled to its own catalytic activity. As a result, its directed polymerization must be driven by the action of an external pH gradient. These polymers appear to lack the functional flexibility of actin.

In sum, actin is a remarkable protein family. Polymer-forming actin appears to be an ancient protein whose ability to generate long-range order and force has been used again and again in evolution to perform many of the tasks that help give cells their dynamic shape and internal organization.

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